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Acute Lymphoblastic Leukemia With Myeloperoxidase Activity

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The French-American-British (FAB) classification of acute leukemias is based on the light microscopic detection of myeloperoxidase (MPO) activity in blast cells. Cells with MPO activity in >3% of cells are classified as acute myeloid leukemia (AML) and usually express myeloid cell surface antigens. We describe a case of acute leukemia in which the blast cells have lymphoid morphology, ultrastructure, immunophenotype, and molecular rearrangements, but express significant amounts of MPO. We discuss the incidence, features, and outcome of MPO-positive acute lymphoblastic leukemia (ALL). © 1996 Wiley-Liss, Inc.

Key words: acute lymphoblastic leukemia, myeloperoxidase, electron microscopy, biphenotypic

INTRODUCTION

The classification of acute leukemias, according to the French-American-British (FAB) system [1], is based on the percentage of cells with myeloperoxidase (MPO) reactivity, positive leukemias being classified as myeloid in origin. Although the existence of mixed lineage and biphenotypic leukemias is well-known, most cases with MPO activity at the light microscope level and expressing lymphoid markers by flow cytometry usually demonstrate other myeloid features in addition to MPO activity. We describe a case of acute leukemia with lymphoid immunophenotype, immunogenotype, and morphology, where the only myeloid feature was significant MPO activity. The typical lymphoid nature resulted in the case being initially diagnosed and treated at a community hospital as a lymphoma.

CASE REPORT

A 33-year-old woman presented to her local physician complaining of fatigue and dizziness. She was noted to be anemic and was managed with red blood cell transfusion without further testing. The patient subsequently developed night sweats and significant weight loss and was referred for further investigation. Physical examination revealed small, generalized lymphadenopathy and minor hepatosplenomegaly. Peripheral blood analysis revealed a hemoglobin level (Hb) of 8.5 g/dl, a platelet count of 66×10^9/l, a raised lactate dehydrogenase (LDH) level of 1,091 units/l (normal range, 0–250), and abnormal liver enzymes. The rest of her hematological and biochemical profile was normal. A bone marrow examination was attempted, but repeated aspirations resulted in dry taps. Bone marrow biopsy revealed the marrow to be 90% cellular and infiltrated by vacuolated cells with a high nuclear:cytoplasmic (N:C) ratio, estimated to be 40% of nucleated cells. A diagnosis of diffuse large cell lymphoma was made. Computerized tomography (CT) scan of the abdomen and pelvis revealed slight hepatomegaly with fatty change in the liver, but no significant intraabdominal lymphadenopathy.

The patient was commenced on treatment with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy. Eight cycles of CHOP were given with initial resolution of her anemia and thrombocyto-

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penia. She had no palpable or radiological disease at the completion of eight cycles, but bone marrow examination revealed persistent abnormal cells, which had the same morphological features as at diagnosis. It was elected to continue with the CHOP chemotherapy but, after two further cycles, abnormal cells, which had the characteristics of lymphoblasts, were noted in the peripheral blood. The LDH level also began to rise rapidly. Repeat bone marrow was 100% cellular with prominent infiltrate of abnormal cells. This specimen was referred for second opinion and was evaluated as being consistent with lymphoblastic lymphoma/leukemia. Because of the significant peripheral blood involvement, the diagnosis of acute lymphoblastic leukemia (ALL) was made, and treatment with hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone) was started. She did not enter remission after the first cycle of induction chemotherapy, and the second cycle, consisting of high-dose methotrexate and cytosine arabinoside, was given. She again failed to achieve remission following this cycle and was referred to the M.D. Anderson Cancer Center for further management.

At referral the patient's white cell count was 36.8×10^9/l with 99% blasts, the hemoglobin level was 9.4 g/dl, and the platelet count was 18×10^9/l. Her electrolytes and renal function were normal but she had an elevated LDH of 25,167 units/l (normal range, 313–618). Bone marrow examination was performed and showed a cellularity of 95% with 97% blasts. The blasts were large with fine, dispersed chromatin and prominent nucleoli. The cytoplasm was basophilic with numerous clear vacuoles. The morphology of the blasts was similar to that of L3 lymphoblastic leukemia (Fig. 1a). Despite this classical lymphoid appearance, 20% of blasts were positive for MPO and Sudan Black at the light microscopy level (Fig. 1b), making the diagnosis, by FAB criteria, that of AML, subtype M1. Periodic Acid-Schiff (PAS) stain was block-positive. Immunofluorescence demonstrated strong terminal deoxynucleotidyl transferase (TdT) positivity in 90% of cells. Electron microscopy was performed and confirmed the presence of MPO in blasts that otherwise had the ultrastructural appearance of L3 or Burkitt-type lymphoblasts (Fig. 1c). Immunophenotyping showed the blasts to be positive for CD10, CD22, and CD19 at 83%, 69.1%, and 85%, respectively, but negative for any myeloid marker, with CD13 being expressed on 5% of cells and CD33 on 2%. T cell markers and human leukocyte antigen (HLA)-DR were negative (7.8%). The blasts did not express surface immunoglobin. Molecular analysis revealed the blasts to have rearrangements of the immunoglobulin heavy-chain gene and T cell receptor delta gene. (Fig. 2a,b). Despite the suggestive morphology, analysis of the c-myc gene revealed it to be in germline configuration (Fig. 2c). Cytogenetic analysis showed the presence of 13 hyperdiploid metaphases with 63–83 chromosomes. Further evaluation was not possible due to the quality of the metaphases.

The patient was treated with Flag-Ida (fludarabine, G-CSF, cytosine arabinoside, and idarubicin), which is the frontline treatment for poor prognosis acute myeloid leukemia (AML) at the M.D. Anderson Cancer Center. She became febrile shortly after admission and was commenced on broad spectrum antibiotics. The patient developed pneumonia during the first week following chemotherapy, and antifungal treatment was added. Following this induction treatment, blasts persisted in the patient's peripheral blood and bone marrow, and a second course of chemotherapy with MOAP (methotrexate, vincristine, PEG asparaginase, and prednisone) was given 25 days after the first course. The patient developed progressive pneumonia, despite multiple antibiotic and antifungal measures, and died of progressive respiratory failure on day 20 of her second cycle.

DISCUSSION

Myeloperoxidase expression in ALL is known to occur, although its significance is not well established [2–7]. This is, to our knowledge, the first report of leukemia with typical ALL morphology with significant MPO activity at the light microscope level in the absence of other myeloid markers. Cases like the one described here, where >3% of cells have detectable MPO activity at the light microscope level, are by definition classified as AML. However, lesser degrees of MPO activity, detected by electron microscopy (EM) are not uncommon in ALL, having been described in 4–8% of ALL cases [2–4]. The presence of MPO by EM is usually correlated with other myeloid markers and may be associated with an adverse prognosis, with a recent study showing comparable rates of remission induction but reduced long-term survival due to short remission duration [5]. The incidence of ALL blasts positive for MPO by mRNA analysis has been reported to be variable, from 0–25% in myeloid marker-negative cases [6,7] to 83% in ALL which coexpresses myeloid surface markers [7,8]. Several cases with mRNA positivity appear to be typical ALL with lymphoid markers and immunoglobulin gene rearrangements and no expression of myeloid markers. The level of mRNA seen in these cells was comparable to the amount in promyelocytic cells, but no MPO protein was detected on Western blot [8]. This may be due to translation defects or contamination of leukemic cells with normal bone marrow cells. The interpretation of mRNA expression will require further investigation.

Although the majority of MPO-positive leukemias, including biphenotypic leukemias, do express myeloid antigens, such as CD13 and CD33, this case does not. This demonstrates that the expression of MPO is not necessarily associated with the expression of other myeloid differentiation features. This may be due to aberrant expression

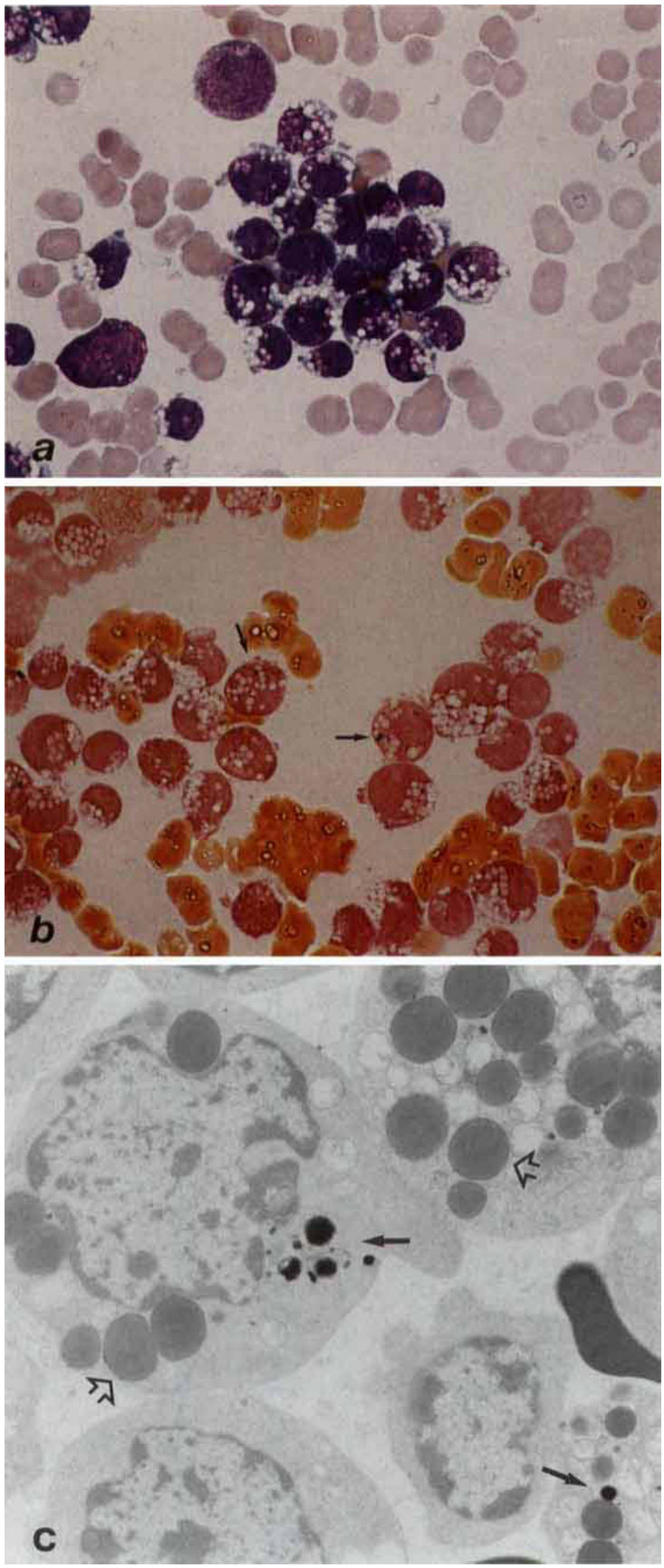
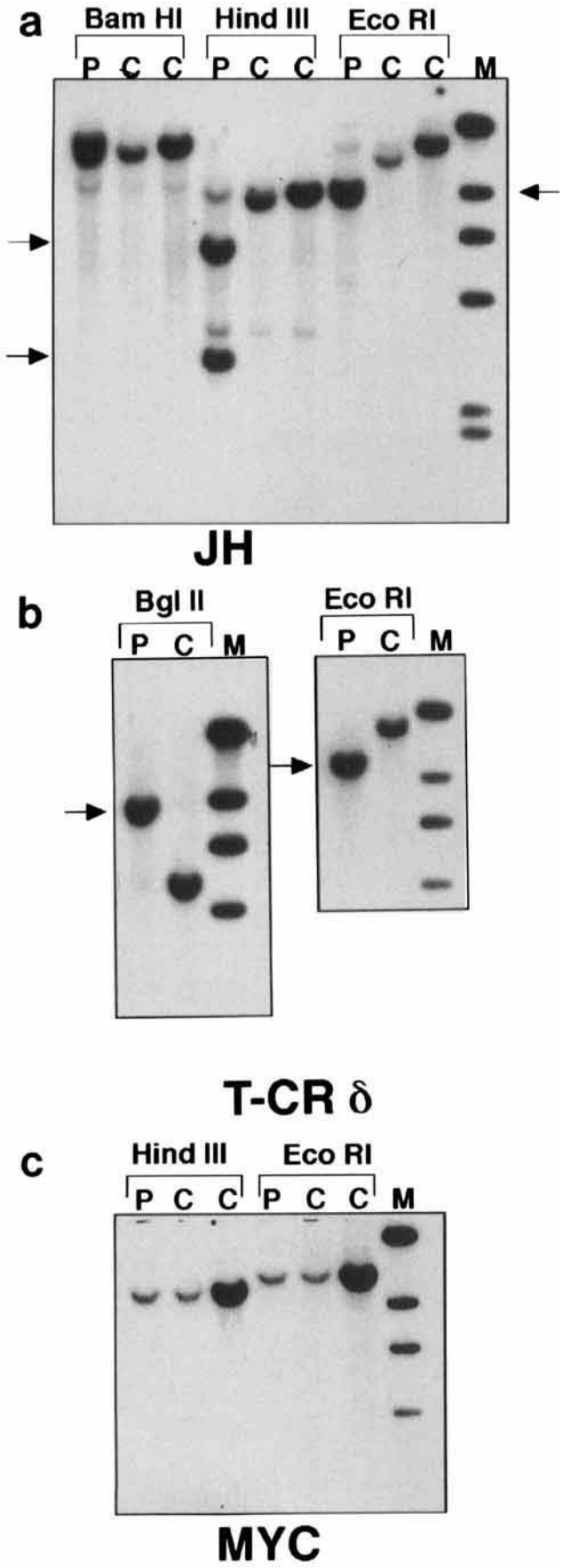


Fig. 1. a: Wright-Geimsa stained smear of bone marrow aspirate, showing blasts with basophilic cytoplasm and multiple vacuoles. b: Myeloperoxidase stain of bone marrow blasts showing positive staining (arrows). There were 20% positive cells. c: Electron microscopic appearance of blasts, showing typical lymphoid morphology, lipid vacuoles (open arrow), and myeloperoxidase-positive granules (solid arrow).



of MPO in a lymphoid progenitor as a result of malignant transformation. Such a malignant phenotype might also result in asynchrony between cytoplasmic maturation and surface antigen expression, causing a myeloid blast to express only MPO. Aberrant expression due to genetic misprogramming is supported by the association of biphenotypic leukemias with certain chromosomal abnormalities, e.g., 11q23 abnormalities. The alternative explanation, however, is that the cell of origin is an early progenitor that retains the capacity to differentiate along both lymphoid and myeloid pathways. This hypothesis is supported by in vitro culture studies, which have shown the ability of such cells to differentiate along several lineage pathways [9]. The finding of MPO mRNA in ALL and in lymphoma and lymphoid leukemia cell lines [10] may indicate that MPO is not as lineage-specific as previously thought.

Although this case must be classified as AML by FAB criteria, the majority of properties such as markers, PAS block-positivity, and gene rearrangements would appear to be more in keeping with ALL expressing myeloperoxidase than TdT-positive AML, which usually expresses other myeloid markers. The morphology was suggestive of Burkett's leukemia/lymphoma, but the lack of surface immunoglobulin supports pre-B ALL. The cytogenetic findings of a hyperdyploid clone of 63–83 chromosomes is also unusual in cases of AML with lymphoid markers, in which cytogenetic abnormalities usually associated with lymphoid malignancies are uncommon [11]. However, the lack of response to hyper-CVAD therapy would be unusual for a lymphoid malignancy. Hyper-CVAD induces complete remission in 95% of previously untreated ALL after two courses [12]. Although this patient had been previously treated, her initial chemotherapy was inadequate for effective treatment of ALL, and hyper-CVAD would have been expected to be effective in the setting of a lymphoid malignancy. As previously mentioned, the literature would suggest that MPO-positive ALL has a comparable rate of remission induction, although long-term survival may be reduced [2,5]. This suggests that, although the biological markers were lymphoid, the behavior of the leukemia was not typical for standard ALL. Whether this is related in some way to MPO positivity is unknown. As previously mentioned, lesser degrees of positivity, such as seen with EM analysis, do not affect remission induction rates. It is likely

Fig. 2. Southern blot analysis of DNA from bone marrow samples of patient (P) probed for JH (a), TCR-d (b), and myc (c). Restriction enzyme digestion, as indicated. Patient (P) DNA was run in conjunction with normal control DNA (C, left) and DNA extracted from HL60 cells (C, right), and a molecular weight marker (M). Rearranged bands in a and b are indicated by arrows.

that the degree of MPO positivity seen in this case reflects a more significant expression of the MPO gene, but the implications of this are unclear.

The patient did not enter remission after treatment with AML-based chemotherapy, either. This may be due to the lower rates of remission induction in AML in general, and perhaps in AML with lymphoid markers or TdT in particular [13–15].

The impact on prognosis of these biphenotypic or mixed-lineage leukemias is not certain, but would appear to be worse than standard AML or ALL. In order to identify prognostic features in these cases it will be necessary to study this group more fully with complete immunophenotypic, ultrastructural, cytogenetic, and molecular analysis. Identification of the biologically important features will allow therapy to be tailored to obtain a better outcome in this subset of leukemias.

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